

REVIEW

by Prof. Dr. Teodora Svetoslavova Handjieva-Darlenska, DSc
Department of Pharmacology and Toxicology, Faculty of Medicine,
Medical University – Sofia

Regarding: the dissertation thesis of Eng. Saveta Georgieva Mladenova entitled **“Investigation of the Anti-Obesogenic Potential of Extracts from *Alchemilla monticola* Opiz and *Ononis spinosa* L. for Obesity Control in In Vitro and In Vivo Models”**, submitted for the award of the educational and scientific degree **“Doctor”**, professional field 5.11 Biotechnology, scientific specialty “Technology of Biologically Active Substances”

1. General presentation of the procedure and the candidate

As a member of the Scientific Jury, I have reviewed the materials submitted in connection with the procedure for awarding the educational and scientific degree “Doctor” to Eng. Saveta Georgieva Mladenova. The dissertation was carried out at the Stephan Angeloff Institute of Microbiology at the Bulgarian Academy of Sciences, under the scientific supervision of Prof. Dr. Milen Ivanov Georgiev.

The dissertation was discussed and approved for public defense by the National Scientific Seminar on “Applied Microbiology and Microbial Biotechnologies” at the Stephan Angeloff Institute of Microbiology – BAS at its meeting held on 19 June 2026. The doctoral candidate was formally discharged with the right to defend her thesis by Order No. I-81/28.05.2026 of the Director of the Stephan Angeloff Institute of Microbiology – BAS.

Eng. Saveta Mladenova holds a Master’s degree in Biotechnology Engineering from the University of Chemical Technology and Metallurgy – Sofia, which she completed in 2003. Her professional development has been closely related to the pharmaceutical and biotechnology industries. Between 2018 and 2022, she was Head of the Development and Innovation Department at BUL BIO – NCIPD Ltd., where she worked on studies related to biotechnological medicinal products, method validation, statistical analysis of test results, and preparation of documentation for regulatory authorities. From 2022 until February 2026, she worked as a Quality Management System Specialist at Comac Medical Ltd. in the field of Phase I–IV clinical trials, and since March 2026 she has held the position of Quality, Research and Development Manager at C7 Commercial Ltd.

This professional background is particularly relevant to the profile of the dissertation. The candidate combines engineering and biotechnology training, experience in pharmaceutical production, quality control, and clinical research with experimental scientific work in the field of biologically active natural substances.

Since 2024, Eng. Mladenova has been a doctoral student at the Metabolomics Laboratory of the Institute of Microbiology – BAS, Plovdiv, in professional field 5.11 Biotechnology, scientific specialty “Technology of Biologically Active Substances”.

2. Relevance and characteristics of the dissertation thesis

The presented dissertation addresses a current and socially significant problem – the search for new natural substances with potential for influencing obesity and the metabolic disorders associated with it.

Obesity is a complex multifactorial disease in which alterations in adipogenesis, lipogenesis, energy balance, and the endocrine function of adipose tissue play a major pathogenetic role. In this context, the development of new approaches capable of modulating adipocyte differentiation and lipid accumulation represents a field of considerable scientific and potential practical interest.

The selection of two plant species – *Alchemilla monticola* Opiz and *Ononis spinosa* L. – is particularly appropriate, as is the investigation not only of their complex extracts but also of individual identified secondary metabolites. In this way, the dissertation goes beyond the conventional description of the biological activity of plant extracts and attempts to clarify specific cellular and molecular mechanisms of action.

The dissertation comprises 108 pages and is illustrated with 24 figures and 7 tables. The bibliography includes 223 references. The structure is logical and includes an introduction, aim and objectives, materials and methods, results, discussion, conclusions, and scientific contributions.

The aim is clearly formulated – to investigate the effects of extracts from *A. monticola* and *O. spinosa* and their secondary metabolites on adipogenesis and lipid accumulation in an in vitro model of human adipocytes, followed by validation of the most significant findings in an in vivo model using *Caenorhabditis elegans*. To achieve this aim, six specific objectives were defined, including phytochemical characterization by NMR-based metabolic profiling, in silico molecular docking, investigation of the anti-adipogenic effects of extracts and individual compounds in human adipocytes, analysis of molecular mechanisms, and validation of the effects of maackiain in an organismal model.

3. Materials, methods and experimental approach

A major strength of the dissertation is the use of a comprehensive methodological approach combining phytochemical, bioinformatic, cellular, molecular-biological, and organismal methods.

The plant extracts were characterized by NMR-based metabolic profiling. In the *A. monticola* extract, a number of flavonoids and other metabolites were identified, including astragalín, catechin, quercetin, quercitrin, isoquercetin, rutin, vitexin, and vanillic acid. In *O. spinosa*, among other compounds, ononin and maackiain were identified and subsequently included in the functional experiments.

Molecular docking was used to assess the potential interactions of selected metabolites with key protein structures involved in adipogenesis and metabolic regulation – PPAR γ , C/EBP α , PI3K, AKT, and AMPK.

Human SGBS preadipocytes were used as the cellular model. This represents an important advantage, since the use of human adipocytes increases the biological relevance of the obtained results. Cell viability, lipid accumulation using Oil Red O staining, lipolysis through determination

of free glycerol, gene expression by RT-qPCR, and protein expression by immunoblot analysis were evaluated.

To validate part of the findings at the organismal level, a model of glucose-induced lipid accumulation in *C. elegans* was used. Lipid accumulation, locomotor activity, viability, and expression of genes involved in energy and lipid metabolism were investigated. Orlistat was used as a reference anti-obesity agent.

4. Results

The results of the dissertation are diverse and, to a large extent, progressively build upon the individual experimental stages.

For *A. monticola*, significant anti-adipogenic activity was demonstrated in human adipocytes. The extract reduced lipid accumulation and increased basal lipolysis. Molecular studies showed inhibition of the PI3K/AKT signaling pathway and negative regulation of the key adipogenic differentiation factors PPAR γ and C/EBP α . Reduced expression of ADIPOQ, SREBP1, ACC, and FASN was also observed, supporting an effect on both adipogenesis and lipogenesis.

The results concerning the individual active components of *A. monticola* are also of interest. Quercitrin significantly reduced lipid accumulation. Astragalin demonstrated a bidirectional, concentration-dependent effect on the PI3K/AKT signaling pathway, with the critical range for the change in effect being between 10 and 25 μ M.

The investigation of *O. spinosa* revealed a more complex pattern. The extract itself did not demonstrate an anti-adipogenic effect identical to that of its individual components, whereas the isolated metabolites ononin and maackiain showed significant activity. Ononin reduced lipid accumulation and affected molecular targets including PI3K and PPAR γ . The proposed mechanism involves an increase in SIRT1 followed by inhibition of PPAR γ .

Particularly interesting are the findings concerning maackiain. In human adipocytes, it reduced lipid accumulation in a concentration-dependent manner, reaching approximately 55% of control values at the highest concentration tested. The data indicate that maackiain inhibits adipogenesis through PPAR γ /C/EBP α signaling and suppresses lipogenesis through SREBP1 and its regulated lipogenic genes.

This part of the work was further validated in *C. elegans*. Maackiain was shown to activate signaling pathways associated with caloric restriction, energy expenditure, and lipid metabolism, including *aak-2/sir-2.1* and *nhr-49/mdt-15*. Increased locomotor activity of the nematodes was also observed, further supporting the possibility of increased energy expenditure. In the experimental model, maackiain demonstrated a favorable anti-obesogenic profile and, for some parameters, showed a superior effect compared with the reference drug orlistat.

5. Scientific contributions

The main fundamental scientific contributions include:

1. Demonstration of the anti-adipogenic potential of *Alchemilla monticola* extract in human adipocytes and clarification of the role of its components astragalin and quercitrin.

2. Elucidation of the role of the PI3K/AKT signaling pathway and PPAR γ in the anti-adipogenic effect of *A. monticola*.
3. Demonstration of a bidirectional, concentration-dependent effect of astragalin on PI3K/AKT signaling.
4. Generation of new data on the anti-adipogenic activity of ononin and proposal of a mechanism involving SIRT1 activation and PPAR γ inhibition.
5. Demonstration of the anti-adipogenic and anti-lipogenic effects of maackiain through PPAR γ /C/EBP α - and SREBP1-dependent mechanisms.
6. First validation of the potential of maackiain to influence lipid metabolism at the organismal level in *C. elegans*.
7. Demonstration of the effect of maackiain on the aak-2/sir-2.1 and nhr-49/mdt-15 signaling pathways, supporting the hypothesis of an action resembling the effects of caloric restriction.

As a scientific and applied contribution, the identified potential of maackiain as a natural biologically active compound that could become the subject of further preclinical investigations in the field of obesity and metabolic disorder control should also be highlighted.

The present results are preclinical and do not allow direct conclusions regarding application in humans, but they provide a solid experimental basis for further studies.

6. Publication activity and scientific achievements

The results of the dissertation have been presented in three scientific publications, in all of which Eng. Saveta Mladenova is the first author. All three articles were published in international Q1 journals:

- *International Journal of Molecular Sciences* – a study on the effects of maackiain and the mimicking of caloric restriction through aak-2-mediated lipid reduction in *C. elegans*;
- *Biomedicine & Pharmacotherapy* – a study on the anti-adipogenic activity of maackiain and ononin and the involvement of PPAR γ in human adipocytes;
- *Frontiers in Pharmacology* – a study on the anti-adipogenic effect of *Alchemilla monticola* through inhibition of PI3K/AKT signaling in human adipocytes.

The combined impact factor of the three publications is 18.388. According to the submitted documentation, the candidate's publications have received 63 citations excluding self-citations in Scopus as of 18 June 2026. This is a particularly good indicator for a doctoral candidate and demonstrates that the obtained results have already attracted attention in the international scientific literature.

The candidate has also participated in six scientific conferences and congresses, with oral and poster presentations of results in the fields of natural products, adipogenesis, and metabolic disorders.

The research included in the dissertation was also carried out with the support of the PlantaSYST project under the European Union's Horizon 2020 programme and a project funded by the European Regional Development Fund through the Operational Programme "Science and Education for Smart Growth", demonstrating the integration of the work into a broader contemporary research infrastructure.

The publication activity of Eng. Mladenova not only meets the requirements for obtaining the educational and scientific degree "Doctor", but clearly exceeds them in terms of journal quality, authorship position, and citation impact.

7. Critical remarks and recommendations

Several remarks can be made regarding the dissertation, although they do not diminish its overall scientific value.

In some parts, the text would benefit from a more concise presentation of the large volume of experimental results and from a clearer distinction between a mechanism that has been experimentally demonstrated and one proposed on the basis of associations between individual molecular parameters.

For some of the findings, particularly those concerning complex plant extracts, it should be kept in mind that the biological effect is likely the result of interactions among multiple components. Therefore, extrapolation of the effects of individual metabolites to the action of the whole extract should be made cautiously.

For future development of this research, I would recommend confirmation of the most interesting findings, particularly those concerning maackiain, in an additional mammalian model of obesity, followed by pharmacokinetic and toxicological studies. This would represent a logical next step toward evaluating the true translational potential of the compound.

8. Conclusion

The dissertation thesis of Eng. Saveta Georgieva Mladenova represents a complete, contemporary, and original scientific study. The topic is relevant, the experimental design is comprehensive, and the methods used allow analysis of the effects of the investigated natural products at different levels – from chemical profiling and in silico modeling to human adipocytes and the whole organism.

I particularly appreciate the combination of different methodological approaches and the attempt to clarify not only the presence of anti-obesogenic activity but also the molecular mechanisms underlying it. The obtained results are of fundamental scientific significance while at the same time outlining opportunities for future applied research.

The scientific output related to the dissertation is of a very good international standard – three publications in Q1 journals, with the candidate as first author in all three, a combined impact factor of 18.388, and substantial citation impact.

Based on the submitted materials, I consider that the dissertation fulfills the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria, the Regulations for its

implementation, and the relevant regulatory requirements for obtaining the educational and scientific degree “Doctor”.

I give my unequivocally positive assessment of the dissertation and recommend that the esteemed Scientific Jury award Eng. Saveta Georgieva Mladenova the educational and scientific degree “Doctor” in professional field 5.11 Biotechnology, scientific specialty “Technology of Biologically Active Substances”.

31 August 2026

Sofia

Prof. Dr. Teodora Svetoslavova Handjieva-Darlenska, DSc